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Low moisture, cooked molasses blocks: A limited intake method for supplementing trace minerals to pre-weaned calves

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ABSTRACT

Two studies were conducted at the Range Cattle Research Education Center (Ona, FL) to evaluate the preference of beef calves for different sources of trace minerals in low moisture cooked molasses blocks (MB; Exp.1) and to evaluate the effects of optimal trace mineral status at weaning while investigating the use of free-choice, MB in a limited intake creep-feeding system (Exp. 2). In Exp. 1, eighteen early-weaned calves (120 d and 185 kg BW) were randomly assigned to 6 bahiagrass pastures (*Paspalum notatum*; n = 3/pasture). Treatments consisted of 3 MB formulations containing 550, 1650 and 2200 mg/kg of Cu, Zn and Mn from 3 different sources (hydroxychloride, organic and sulfate). Calves had continuous and simultaneous access to each of the 3 MB treatments. Intake was greatest ($P = 0.04$) for MB fortified with hydroxychloride and was similar between organic and sulfate (0.35, 0.27, and 0.28 kg/d, respectively). In Exp. 2, forty-eight cows with heifer calves (n = 24 pairs/year) were randomly assigned to 12 bahiagrass pastures (n = 2 cow-calf pairs / pasture). Treatments consisted of 2 MB formulations; (1) Control = no added minerals; or (2) Fortified = 550, 1650, and 2200 mg/kg of Cu, Zn, and Mn from hydroxychloride sources. Sodium, Ca, Se, Co, and I were formulated into the Fortified MB treatment at levels to meet NASEM recommendations. Following weaning, calves were transferred to fully covered individual pens for a 30-d evaluation. Molasses block intake was greater ($P = 0.01$) among calves assigned to Control vs. Fortified treatment (0.35 vs 0.21 kg/d). A treatment × year effect was detected ($P \leq 0.01$) for liver trace mineral concentrations, where calves assigned to Fortified vs. Control treatment had greater concentrations of Co, Cu, Mn, and Se at weaning in year 1, but not in year 2. For the Fortified treatment, MB intake was 38 % less in year 1 vs. 2, which correlated with less liver Cu ($r = 0.52$; $P < 0.01$) and Se ($r = 0.79$; $P < 0.01$). In summary, MB supplements, fortified with hydroxychloride sources of Cu, Zn, and Mn, are preferred by calves when compared to other mineral sources. The supplementation of pre-weaned calves with mineral-fortified low moisture, cooked MB was effective as a limit-feeding vehicle for trace mineral delivery; however, calf mineral status at weaning is dependent upon adequate supplement intake.

Abbreviations: ADG, average daily gain; BW, body weight; DMI, dry matter intake; MB, low moisture cooked molasses blocks.

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1. Introduction

Weaning of beef calves is one of the most stressful events in a calf's life. The transition into a new production system (i.e. stocker or feedlot) often results in changes in physiological, immunological, and nutritional status. The disturbances observed in this period are associated with stressful practices around weaning, which usually involves physical and physiological stressors such as dehorning, deworming, handling, shipping, and commingling with a new group of calves (Grandin, 1997). Encountering these stressors can disrupt normal endocrine and neuroendocrine function and adversely affect performance. Negative effects of some of these stressors can be prevented or overcome through management practices and nutritional strategies (Carroll and Forsberg, 2007). A common strategy aiming to reduce negative effects associated with weaning and feedlot entry is the use of creep-feeding, by proving calves with early human interaction and nutritional support (Lardy and Maddock, 2007). Calves provided pre-weaning trace mineral supplementation through limit-fed creep feeding (Caramalac et al., 2017) or injectable trace mineral (Arthington et al., 2014) had greater trace mineral status and heightened inflammatory responsiveness at weaning. Nevertheless, logistics associated with creep-feeding supplementation can limit the success of this strategy, whereas low moisture cooked MB fortified with trace minerals may be used to conveniently provide pre-weaning calf trace mineral supplementation in cow-exclusion areas, reducing cost and labor associated with feeding.

We hypothesized that calves will have improved pre-weaning body weight (BW) gain, greater mineral status at weaning, and heightened post-weaning inflammatory responsiveness when provided free-choice access to trace mineral-fortified MB prior to weaning compared to calves supplemented with MB without mineral fortification. Further, we hypothesized that when given the opportunity to choose, calves will prefer MB fortified with hydroxychloride sources of Cu, Zn, and Mn when compared to sulfate and organic sources of the same minerals. Thus, the objectives of this study were to evaluate preferences of beef calves for different sources of trace minerals in low moisture cooked MB (Exp.1), and to evaluate calf performance before and after weaning, thus improving knowledge pertaining to the value of optimal trace mineral status at the time of weaning while investigating the utility of using free-choice, MB in a limited intake creep-feeding system (Exp. 2).

2. Materials and methods

Two experiments were conducted at the University of Florida – IFAS, Range Cattle Research and Education Center (Ona, FL) to evaluate preferences for mineral sources and effects of pre-weaning supplementation of calves using free-choice, MB with or without mineral fortification. All procedures were approved by the Institutional Animal Care and Use Committee of University of Florida (protocol #201,810,315).

2.1. Low moisture cooked molasses blocks manufacturing

Molasses blocks were manufactured at the Kansas State University Beef Cattle Research Center in Manhattan, Kansas (USA).

Blocks were produced by an evaporative process using a 60-L steam-jacketed, cone-bottomed kettle equipped with a scraped-surface agitator rotating at 60 revolutions per minute. Temperature and pressure within the vessel were monitored and recorded at 5-second intervals using indwelling probes. Sugarcane molasses (27.5 kg) and food-grade corn oil (1 kg) were added to the kettle under continuous agitation. The amount of wet molasses added was based on an assumed shrink (due to evaporative loss of moisture during processing) of 22 %. Steam flow through the outer jacket was initiated, heating contents of the kettle for approximately 15 min to form a viscous mass with a final temperature of 138 °C. A blend (2.5 kg) of ground limestone and the appropriate trace mineral premix was then added to the kettle and mixed for 2 min. The kettle was then sealed, and vacuum was applied for 15 s with continuous agitation, causing contents to expand by two to three volumes, followed by release of the vacuum and an additional 15 s of agitation. The vacuum cycle was repeated three times to facilitate thorough mixing of components. The vessel was then sealed, steam flow was suspended, and internal pressure was reduced to −90 kPa, effectively decreasing temperature of the contents to 88 °C within 2 min and reducing moisture content of the mix to approximately 2%. The vacuum was then released, a discharge valve located at the bottom of the vessel was opened, and the vessel was pressurized to discharge contents into a tared high-density polyethylene container placed below the discharge port. A polyethylene sheet (5 mm thickness) was applied directly to the surface of the mixture to prevent assimilation of atmospheric moisture, and a rigid plastic cover was placed onto the container to facilitate stacking. Blocks were then weighed to determine mass balance. Between batches, the vessel was filled with water, contents were heated under continuous agitation for 3−4 min, and then discarded, thus minimizing cross-contamination between treatments. Blocks were cooled at ambient temperature for 24 h before stacking onto pallets and shipping to the study site. Final composition of the blocks was approximately 4% oil, 10 % mineral premix, and 86 % evaporated molasses. Actual recovery of finished products was 99 % or more of theoretical mass.

2.2. Experiment 1 - Animals, diets, handling, and study design

The effects of mineral fortification with different sources of Cu, Zn, and Mn on preferential consumption of MB were evaluated in a 6-week period.

Eighteen early-weaned calves (Brahman × British; 120 d and 185 kg BW) were randomly assigned to six bahiagrass (*Paspalum notatum*; n = 3 calves and 0.23 ha/paddock) pastures. Treatments consisted of molasses blocks formulations contained 550, 1650 and 2200 mg/kg of Cu, Zn and Mn, respectively, from 3 different sources (hydroxychlorides, organics, or sulfates). Sodium, Ca, Se, Co and I were formulated at the same inclusion level for all treatments to meet NASEM recommendations (NASEM, 2016) at the targeted level of consumption. Calves were provided concentrate supplement daily at 20 g/kg of BW (218 g/kg CP and 730 g/kg TDN) and had free access to white stock salt.

Calves in each pasture had continuous and simultaneous access to each of the three MB treatments (1 block from each treatment, 3 blocks/pasture). Preferential intake was estimated by determining weekly disappearance rate (i.e., weight change) of the MB. Individual intakes were estimated by dividing total intake within a pasture by the number of calves (n = 3) and expressed as daily intake. Molasses blocks were replaced with a new block of the same treatment when block weight was ≤ 3 kg.

2.3. Experiment 2 - Animals, diets, handling, and study design

A total of forty-eight cows with heifer calves (Brahman × British; n = 24 pairs/year) were randomly assigned to 12 established bahiagrass pastures (n = 2 cow-calf pairs/pasture) with free-choice access to water and supplemental white stock salt with no added minerals. Calves were provided pre-weaning supplements within 6 m^2 cow-exclusion areas for 86 and 92 d (approximately 13 weeks) in year 1 and 2, respectively. Treatments consisted of two MB formulations; (1) Control = no added minerals; or (2) Fortified = 550, 1650, and 2200 mg/kg of Cu, Zn, and Mn from hydroxychloride sources. Sodium, Ca, Se, Co, and I were also formulated into the Fortified block treatment at levels to meet NASEM recommendations (NASEM, 2016) at the targeted level of intake. Calves had continuous, free-choice access to MB throughout the study. Molasses block consumption was estimated by disappearance rate (i.e weight change) of the blocks which were measured weekly and replaced when block weight was ≤ 3 kg. Further, Cu and Se intakes (mg/d) were estimated using calculated block consumption and block nutritional analysis of both elements.

Immediately following weaning, calves were transferred to fully covered individual drylot pens (10 m^2/pen) for a 30-d evalution. During the post-weaning period, calves were provided free-choice access to ground bermudagrass (*Cynodon dactylon)* hay and a grain-based concentrate offered in separate individual feeding spaces, thus providing an ability to measure intake selection. Post-weaning dry matter intake (DMI) of concentrate and hay were estimated daily individually for each calf by subtracting the dry matter of the daily refusal from the dry matter of the daily offer. Concentrate and hay intakes were determined individually for each calf but were summarized and presented in 10 intervals of three days each as the average daily intake.

Calf BW at the start of the study, at weaning, and on d 15 and 30 after weaning were measured following a 16-h period of feed and water withdrawal. For initial BW, calves were removed from pasture but remained with their dams over the 16-h period.

2.4. Blood sampling, liver biopsy, and laboratory analyses (Exp. 2)

Liver tissue was collected at weaning by a trained technician using techniques previously described (Arthington and Corah, 1995). Samples were collected between the 11th and 12th intercostal space using a Tru-Cut biopsy needle (CareFusion, 14-gauge x15 cm; Becton Dickinson, Vernon Hills, IL, USA). Four core tissue samples were collected from each animal. Following collection, samples were frozen at -20 °C, and sent to an analytical laboratory for mineral analyses (Michigan State University, Animal Health Diagnostic Laboratory, Lansing, MI, USA).

Blood samples were collected from the jugular vein on d 0, 1, 4, 7, 11, 14, and 33 relative to weaning to determine plasma concentrations of cortisol, haptoglobin, and ceruloplasmin (markers of acute phase response). Blood samples were collected into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ, USA) containing 158 USP units of freeze-dried sodium heparin. Blood samples were placed on ice immediately following collection and centrifuged at 1200 × g for 30 min at 4 °C for recovery of plasma. Plasma samples were frozen at −20 °C in the same day of collection.

Plasma haptoglobin concentrations were determined in duplicate samples by a biochemical assay measuring haptoglobin-hemoglobin complexing by the estimation of differences in peroxidase activity (Makimura and Suzuki, 1982). Results were obtained as arbitrary units resulting from the absorption reading at 450 nm. Same quality control standards used in the biochemical assay were analyzed by quantitative determination of bovine haptoglobin in plasma (bovine haptoglobin ELISA test kit; Life Diagnostics, Inc., West Chester, PA, USA). The concentrations of haptoglobin, based on the ELISA assay, ranged from 0.03 (low control) to 0.95 mg/ml (high control) with an intra-assay CV of 1.26 %. The ELISA standard curve was used to convert the arbitrary units obtained from the biochemical procedures into mg/mL (Cooke and Arthington, 2013) with the least detectable value of 0.03 mg/mL. Inter-assay CV were 5.9 and 6.0 % and intra-assay CV were 6.2 and 7.9 % for years 1 and 2, respectively.

Plasma ceruloplasmin oxidase activity was measured in duplicate samples using colorimetric procedures described by Demetriou et al. (1974). Ceruloplasmin concentrations are expressed as mg/dL as described by King (1965). Inter-assay CV were 2.2 and 1.76 % and intra-assay CV were 4.6 and 6.0 % for years 1 and 2, respectively.

Plasma cortisol concentrations were measured using a chemiluminescent enzyme immunoassay (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). Intra-assay CV was 3.2 and 5.8 % for year 1 and 2, respectively.

2.5. Molasses block, forage, hay, and concentrate sampling and analysis

Nutritional analysis of MB, pastures, hay, and concentrate used in Exp. 2 were analyzed individually in each year (Table 1).

Duplicate samples were collected from 3 random MB of each treatment. Samples were collected by slowly pouring liquid nitrogen while coring the material with a high-speed electric drill (1.9 cm bit attachment). The liquid nitrogen was used to prevent the material from melting due to the heat of the drilling process. Following collection, samples were immediately frozen. Samples were analyzed in duplicate in a commercial laboratory (Dairy One Forage Laboratory; Ithaca, NY, USA).

Forage samples from each of the 12 pastures used during the supplementation phase of Exp. 2, were hand-plucked at the beginning of the study in each year. Samples of the 12 pastures were composited, and dried in a forced-air oven at 60 °C for 72 h. The composite samples were ground in a Wiley mill (Model 4, Thomas-Wiley Laboratory Mill, Thomas Scientific, Swedesboro, NJ, USA) to pass a 4-mm stainless steel screen. Samples were analyzed for of all nutrients in duplicate in a commercial laboratory (Dairy One Forage Laboratory; Ithaca, NY, USA).

Hay and concentrate samples were randomly collected at the beginning of the post-weaning phase of Exp. 2. Samples were dried in a forced-air oven at 60 °C for 72 h. Samples were ground in a Wiley mill (Model 4, Thomas-Wiley Laboratory Mill, Thomas Scientific, Swedesboro, NJ, USA) to pass a 4-mm stainless steel screen. Samples were analyzed for all nutrients, in duplicate in a commercial laboratory (Dairy One Forage Laboratory; Ithaca, NY, USA).

2.6. Statistical analysis

All data were analyzed using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC; Version 9.4). In Exp. 1, block intake was analyzed using pasture as the experimental unit. Model statements included fixed effects of treatment, week, and treatment × week, and random effect of pasture (treatment). In Exp. 2, pasture was the experimental unit for block intake and mineral intake for the pre-weaning phase. The model statement included treatment, week, year, and the possible interactions with week in the repeated statement. For initial and weaning BW, liver mineral concentrations, and average daily gain (ADG) measurements, pasture was the experimental unit and the model statement included fixed effects of treatment, year, and their interaction, and pasture within treatment × year as a random effect. Further, Pearson correlations were conducted to evaluate the linear relationships between mineral intake and mineral status at weaning.

For the post-weaning phase, calf was the experimental unit for DMI. The model statement included fixed effects of treatment, interval, year, and all possible interactions, and calf (pasture) and pasture (treatment × year) as random effects. For the BW measurements calf was the experimental unit and model statements included fixed effects of treatment, year, the interaction, and random effect of pasture (treatment × year). Blood measurements were analyzed as repeated measures, where calf was the experimental unit and the model statement included treatment, day, and year. Compound symmetry covariance structure was used for the repeated-measures analyses, as this covariance structure generated the lowest Akaike information criterion.

Data were separated using PDIFF if a significant preliminary F-test was detected. Significance was set at $P \leq 0.05$, and tendencies if $P > 0.05$ and ≤ 0.10.

3. Results

3.1. Block preferential intake

Treatment ($P = 0.01$) and week ($P = 0.01$), but not treatment × week effects, were detected for block disappearance rate. Block disappearance rate was greatest ($P = 0.04$) for MB fortified with hydroxychloride sources of Cu, Zn, and Mn, and was similar between organic and sulfate sources of the same minerals (0.35, 0.27, and 0.28 kg/d, respectively). Total block intake was greater ($P = 0.03$) on weeks 2, 4, and 5 of the evaluation period (0.38, 0.33, and 0.35 kg/d, respectively), compared to weeks 1, 3, and 6 (0.27, 0.27, and 0.26 kg/d, respectively; data not shown).

Table 1
Chemical composition of low moisture cooked MB, pasture, hay and grain used in Exp. 2.[1]

Item	Control Block	Fortified Block	Pasture	Hay	Concentrate	Control Block	Fortified Block	Pasture	Hay	Concentrate
	Year. 1					*Year. 2*				
CP, g/kg	63	58	111	91	218	62	64	135	114	262
ADF, g/kg	.	.	386	364	304	.	.	362	441	223
NDF, g/kg	9	15	707	729	444	.	.	722	783	362
NFC, g/kg	.	.	75	81	.	.	.	35	4	.
TDN, g/kg	898	893	575	535	730	.	.	575	525	760
Ca, g/kg	9	41	5	6	9	52	44	4	3	13
P, g/kg	1	1	2	3	5	1	1	2	3	6
Mg, g/kg	4	4	3	4	3	4	4	4	2	3
K, g/kg	47	42	12	8	15	45	46	14	15	15
Na, g/kg	1	1	0	0	2	1	1	0	0	1
Fe, mg/kg	176.5	485.8	106.5	95.0	164.5	406.3	541.8	87.0	75.0	188.5
Zn, mg/kg	12.3	1625.0	27.0	43.5	44.5	17.0	1730.0	24.5	48.0	56.0
Cu, mg/kg	7.3	548.5	7.5	8.5	8.0	6.8	589.5	7.0	9.0	9.0
Mn, mg/kg	26.0	2067.5	28.0	36.5	22.5	42.0	2177.5	33.0	69.0	28.0
Mo, mg/kg	2.3	1.9	1.2	0.8	3.0	2.1	2.1	1.2	1.5	3.5
S, g/kg	7	6	2	3	4	7	8	0.2	0.2	0.4
Se, mg/kg	1.2	26.3	0.0	0.1	0.2	1.2	24.8	0.0	0.0	0.2

[1] MB = low moisture cooked molasses blocks; CP = crude protein; ADF = acid detergent fiber; NDF = neutral detergent fiber; NFC = non fiber carbohydrates; TDN = total digestible nutrients; Ca = calcium; P = phosphorus; Mg = magnesium; K = potassium; Na = sodium; Fe = iron; Zn = zinc; Cu = copper; Mn = manganese; Mo = molybdenum; S = sulfur; Se = selenium.

3.2. Pre-weaning block intake, calf body weight and average daily gain

Effects of treatment ($P = 0.01$), week ($P < 0.01$), year ($P < 0.01$), and week × year ($P < 0.01$) were observed for block intake. Voluntary MB intake was 40 % less ($P = 0.01$) when fortified with minerals (0.35 vs 0.21 kg/d, respectively for Control vs. Fortified). Overall, calves voluntarily consumed 29 % less ($P = 0.01$) block supplement in year 2 vs. 1. Within treatment, this reduction was 38 and 25 % for Fortified and Control treatments, respectively. These differences varied by week with MB intake greater ($P \leq 0.02$) on weeks 2, 6, and 12 in year 1 vs. year 2 (Fig. 1).

Initial calf BW did not differ at the start of the pre-weaning supplementation period. Despite differences in block intake, no treatment differences were observed for pre-weaning ADG. A year effect was observed, however, where calves in year 2 had greater ($P < 0.01$) ADG than year 1. No effects of treatment × year were observed. Similarly, no treatment differences were observed for weaning weight. Due to greater ADG among all calves in year 2, a year effect was observed ($P < 0.01$), where calves in year 2 were heavier at weaning than calves in year 1 (Table 2).

3.3. Mineral status and mineral intake

A treatment × year effect was detected ($P \leq 0.01$) for liver concentrations of Co, Cu, Mn, and Se at weaning. Calves assigned to Fortified vs. Control treatment had greater ($P < 0.01$) liver concentrations of Co, Cu, Mn, and Se at weaning in year 1, but not in year 2 (Table 3).

Effects of treatment ($P < 0.01$), week ($P < 0.01$), year ($P < 0.01$), and all the possible interactions ($P < 0.01$) were observed. As per experimental design, a treatment effect was expected for mineral intake, whereas effects of week, year, and the interactions with treatment are explained by the differences in block intake observed over individual weeks within years. Calves assigned to the Fortified treatment had 31 and 39 % less Cu and Se intake in year 2 vs. 1 (145 and 99, and 6.9 and 4.2 mg/d of Cu and Se in year 1 and 2, respectively) explaining the differences observed in mineral status at weaning in both years of the study.

3.4. Post-weaning dry matter intake

Effects of treatment ($P < 0.01$), interval ($P < 0.01$), year ($P < 0.01$), interval × year ($P < 0.01$), and treatment × interval × year ($P = 0.01$) were observed for total DMI. In year 1, calves assigned to Fortified treatment had greater ($P \leq 0.02$) total DMI as a percentage of BW from interval 6–10 (d 16–30 post-weaning) when compared to calves assigned to Control treatment. In year 2, calves assigned to Fortified treatment had greater ($P \leq 0.05$) total DMI as a percentage of BW in intervals 9 and 10 (d 25–30 post-weaning) when compared to calves assigned to Control treatment (Fig. 2).

Effects of interval ($P < 0.01$), year ($P < 0.01$), interval × year ($P < 0.01$), interval × treatment ($P = 0.03$) and treatment × interval × year ($P < 0.01$), but no effects of treatment were observed for concentrate intake as a percentage of BW in the post-weaning phase. In year 1, but not in year 2, calves assigned to Fortified treatment had greater ($P < 0.01$) concentrate intake as percentage of BW on intervals 7–10 (d 19–30 post-weaning) when compared to calves assigned to Control treatment (Fig. 3).

Effects of treatment ($P < 0.01$), interval ($P < 0.01$), year ($P < 0.01$), and interval × year ($P < 0.01$) were observed for hay intake as percentage of BW in the post-weaning phase, where hay intake was greater ($P < 0.01$) for calves assigned to Fortified vs. Control

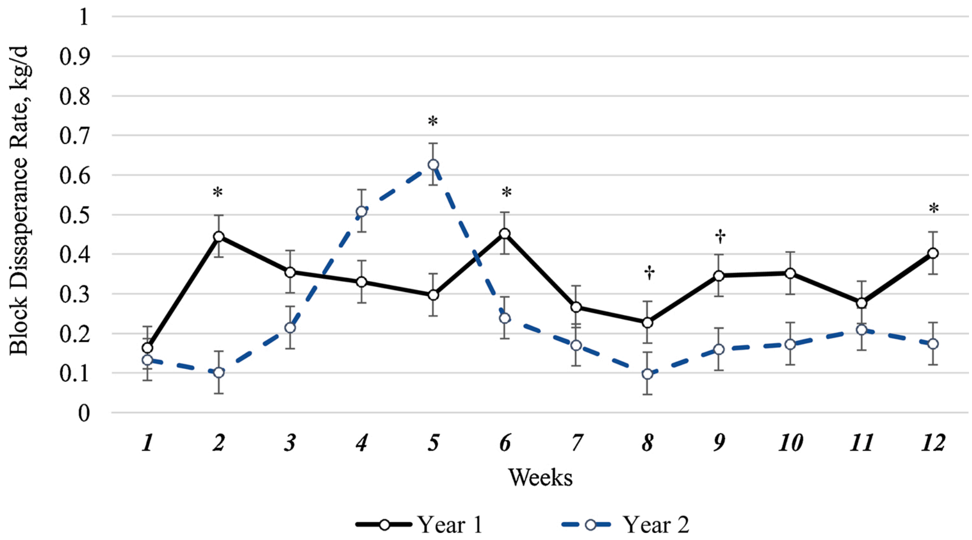

Fig. 1. Calf voluntary intake (kg/d) of low moisture cooked molasses blocks (**MB**) among pre-weaned calves over 12 weeks prior to weaning in year 1 and year 2 in Exp. 2. A week × year ($P < 0.01$) was observed. Block intake was greater (*; $P \leq 0.02$) on weeks 2, 6, and 12 and tended to be greater (†; $P \leq 0.09$) on weeks 8 and 9 in year 1 vs. year 2. Further, block intake on week 5 was greater ($P < 0.01$) in year 2 than year 1.

Table 2
Pre and post weaning performance of calves supplemented with MB with (fortified) or without (control) trace mineral supplementation prior to weaning in Exp. 2.[1].

Item	Control	Fortified	SEM	P-value
Initial BW, kg	190	185	3.9	0.36
Weaning BW, kg	264	258	4.2	0.38
Pre-weaning ADG, kg/d	0.84	0.84	0.036	0.97
BW d 15 post-weaning, kg	250	248	5.8	0.75
Final BW, kg	272	262	5.5	0.22
Overall ADG, kg/d	0.20	0.05	0.10	0.30
	Year 1	Year 2	SEM	P-value
Initial BW, kg	188	186	3.9	0.61
Weaning BW, kg	245	277	4.2	< 0.01
Pre-weaning ADG, kg/d	0.66	1.02	0.036	< 0.01
BW d 15 post-weaning, kg	238	260	5.8	0.02
Final BW, kg	241	293	5.5	< 0.01
Overall ADG, kg/d	−0.14	0.39	0.10	< 0.01

[1] MB = low moisture cooked molasses blocks; SEM = standard error of the mean; BW = body weight; ADG = average daily gain.

Table 3
Liver mineral concentration of calves supplemented with low moisture cooked MB with (fortified) or without (control) trace mineral fortification prior to weaning in Exp. 2.[1].

Mineral	Year	Control	Fortified	SEM	Trt P - value	Trt x Year P - value
		mg/kg dry matter				
Cu	1	86	169	19.7	< 0.01	0.01
	2	47	44	19.7	0.92	
Se	1	0.49	1.2	0.12	< 0.01	0.01
	2	0.21	0.40	0.12	0.15	
Co	1	0.11	0.37	0.037	< 0.01	< 0.01
	2	0.11	0.10	0.037	0.75	
Mn	1	6.1	7.8	0.64	0.01	< 0.01
	2	3.6	2.4	0.64	0.06	
Zn	–	153	162	7.2	0.39	–

[1] MB = low moisture cooked molasses blocks; SEM = standard error of the mean; Trt = treatment; Cu = copper; Se = selenium; Co = cobalt; Mn = manganese; Zn = zinc.

treatments. Irrespective of treatment, hay intake as a percentage of BW was greater ($P < 0.01$) in intervals 1 and 2 in year 2 vs. 1. However, hay intake as a percentage of BW was greater ($P < 0.01$) from interval 3–10 in year 1 vs. 2 (Fig. 4).

3.5. Post-weaning body weight

An effect of year ($P = 0.02$) was observed for calf BW on d 15 post weaning, where calves in year 2 were heavier than calves in year 1 (260 and 238, respectively). No treatment differences were observed for calf BW on d 15 post-weaning.

Effects of treatment × year ($P = 0.04$), year ($P = 0.02$) but not treatment were observed for ADG in the first 15 d of the post-weaning phase. In year 1 but not in year 2, calves assigned to Control treatment had less ($P = 0.04$) ADG than calves assigned to the Fortified treatment (0.02 vs. 1.00 and -0.09 vs -0.51 kg/d in year 1 and 2, respectively).

A year effect ($P < 0.01$) was observed for final BW, where calves in year 2 had greater final BW than calves in year 1 (241 vs. 293, respectively). No treatment differences were observed for final BW.

Similarly, a year effect ($P < 0.01$) was observed for overall ADG where calves in year 2 had greater ADG than calves in year 1 (-0.14 and 0.39 kg/d, respectively). No treatment effects were observed for overall ADG (Table 2).

3.6. Acute phase response

A day × year effect ($P = 0.01$) was observed for plasma haptoglobin, but no effects of treatment or treatment x day were observed. Plasma haptoglobin concentrations peaked on d 4 for both years, but in year 1, concentrations increased more rapidly resulting in a tendency ($P = 0.07$) for greater plasma haptoglobin concentrations on d 1 following weaning in year 1 vs. 2 (Fig. 5).

Treatment × day × year ($P = 0.01$) and day × year ($P < 0.01$) effects, but not treatment or treatment × day effects, were observed for plasma ceruloplasmin. Concentrations of plasma ceruloplasmin increased ($P < 0.01$) from d 0 (weaning) and peaked on d 7 for both treatments in year 1. However, in year 2 plasma ceruloplasmin concentration of calves assigned to Control treatment peaked on d 7 while plasma ceruloplasmin concentration of calves assigned to Fortified treatment peaked on d 1. Plasma ceruloplasmin concentrations were greater ($P < 0.01$) on d 7 and 11 in year 2 than year 1 (Fig. 6).

Effects of day × year ($P < 0.01$) were observed for plasma cortisol concentration. Plasma cortisol concentrations were largely

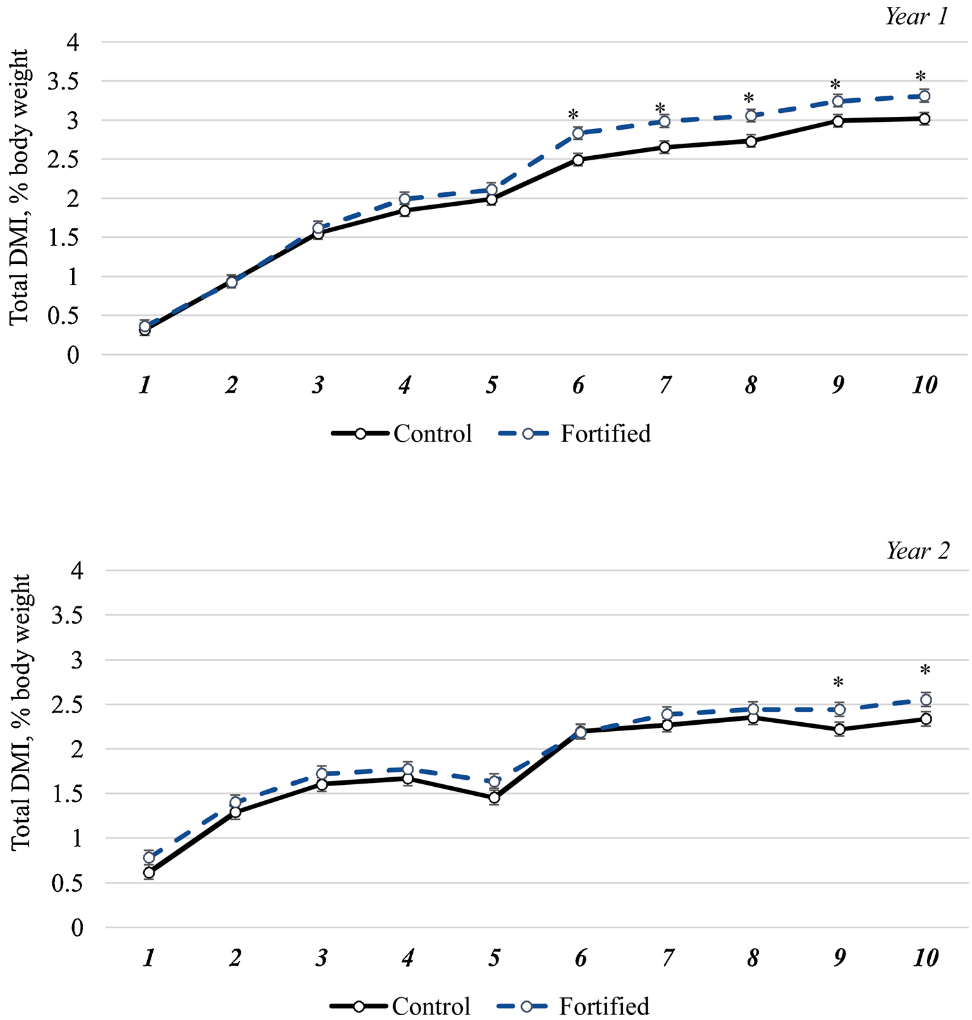

Fig. 2. Total dry matter intake (**DMI**) as percentage of body weight (**BW**) during the post-weaning phase in Exp. 2. Data is presented as the daily average intake for each interval which consisted of 3 consecutive days. Effects of interval x treatment x year ($P = 0.01$) were observed. * Calves assigned to Fortified treatment had great total DMI as percentage of BW than calves assigned to Control treatment ($P \leq 0.05$).

driven by d 0 where calves in year 1 had greater plasma cortisol concentrations than calves in year 2 (Fig. 7). Additionally, calves assigned to Fortified treatment had greater ($P = 0.05$) overall plasma cortisol concentration than calves assigned to Control treatment (2.14 vs 2.50 μg/mL)

4. Discussion

The use of self-fed supplements such as MB, is a convenient method to address nutrient deficiencies of forages, such as trace mineral deficiencies. Further, the use of self-fed supplements is also known to reduce labor associated with feed delivery and feeding costs (Kunkle et al., 2000). As previously observed (Caramalac et al., 2017), calves exhibit preferential intake for hydroxychloride source when offered supplements fortified with different sources of Cu, Zn, and Mn. In Exp. 1, calves preferred supplements fortified with hydroxychloride sources of Cu, Zn, and Mn compared to supplements fortified with organic and sulfate sources of the same minerals. One possible explanation for greater preferential intake of MB fortified with hydroxychloride sources of Cu, Zn, and Mn is related to the low solubility of these mineral sources at neutral pH, as demonstrated by Cao et al. (2000) and Guo et al. (2001). The greater solubility of the sulfate and organic sources could result in a “metallic-like” taste, resulting in decreased supplement intake due to feed aversion. Although mineral source appears to impact preferential intake of supplements, the simple presence of minerals, regardless of source, impacts overall free-choice supplement intake. In Exp 2, free-choice intake of MB was 40 % less when minerals were added to the supplement compared to blocks with no added mineral, confirming the effects of mineral inclusion on feed palatability. This resulted in 140 g/d of greater supplement intake for calves assigned to the Control vs. Fortified treatment. Although MB intake was affected by mineral fortification, no treatment differences were observed in pre-weaning ADG or weaning weight. Similarly, no differences on

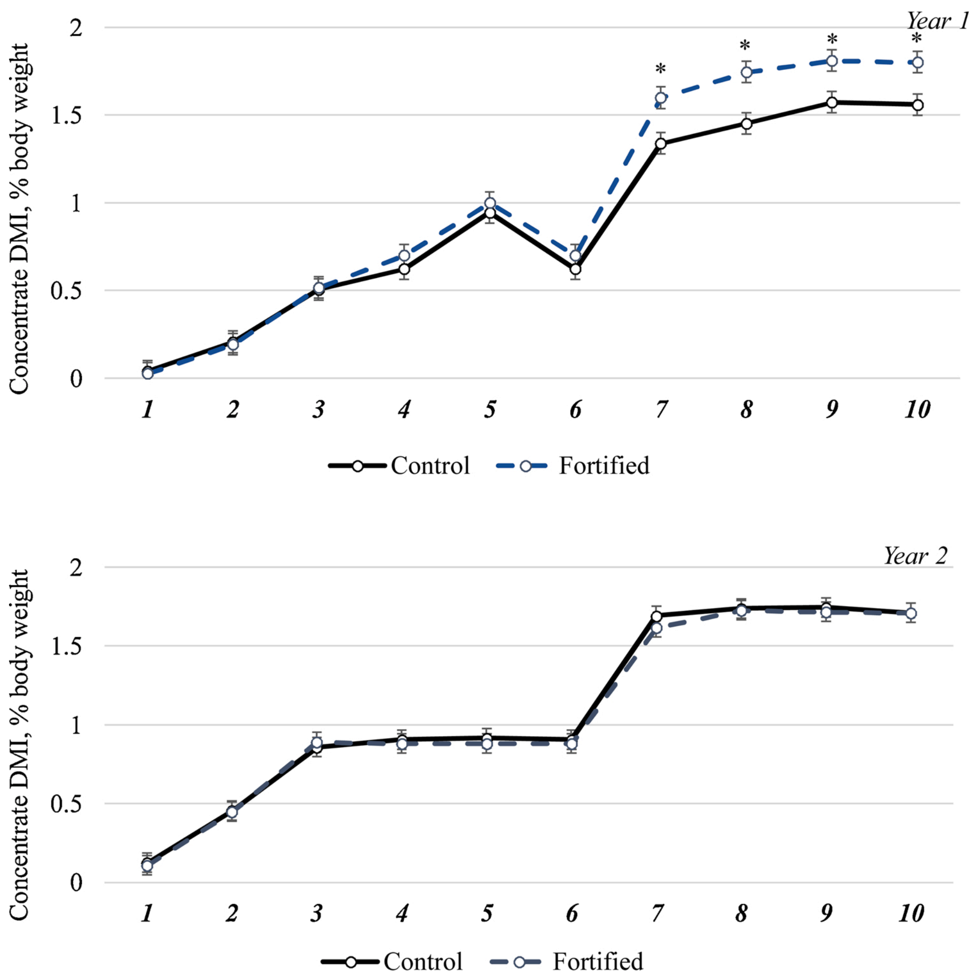

Fig. 3. Concentrate dry matter intake (**DMI**) as percentage of body weight (**BW**) during the post-weaning phase in Exp. 2. Data is presented as the daily average intake for each interval which consisted of 3 consecutive days. There was no effect of treatment, but an interval x treatment x year effect ($P < 0.01$) was observed. * In year 1, calves assigned to Fortified treatment had great DMI than calves assigned to Control treatment ($P < 0.01$).

weaning weight were observed by Caramalac et al. (2017) when supplementing calves with or without mineral fortification prior to weaning. Moriel and Arthington (2013) also reported no differences in weaning weight of calves consuming mineral fortified supplement prior to weaning. The lack of supplementation effects on weaning weights could be explained by a poor efficiency of BW gain as suggested by Stricker et al. (1979) which is often observed with unlimited access to creep-feed. Alternatively, Faulkner et al. (1994) reported improved weaning weight with limited-fed creep feeding strategies (< 1.0 kg/d), which could be expected when considering the limited intake observed in this study (approximately 0.28 kg/d). However, although intake was fairly low (as observed with sugar cane molasses-based supplements) is likely that the variation on daily intake, which has been discussed by Bowman and Sowell (1997) negatively impacted weaning weights.

At weaning in year 1, calves assigned to the Fortified treatment had greater mineral status of Co, Cu, Mn, and Se when compared to calves assigned to the Control treatment. Similar results were reported previously (Caramalac et al., 2017) where calves were provided grain-based supplements fortified, or not, with trace minerals for approximately 84 d prior to weaning in a creep-feeding system. In contrast, in year 2, no differences in mineral status at weaning were observed between treatments. In fact, in year 2, calves were considered deficient or marginally deficient for Cu ($\leq$ 40 mg/kg) and Se ($\leq$ 0.6 mg/kg) at the time of weaning for both treatments. This lack of treatment effect is likely due to a 38 % lesser voluntary intake of the Fortified supplement in year 2 vs. 1, which resulted in less intake of Cu and Se. Further, this reduction in Cu and Se intake reflected in the mineral status of calves at weaning, which is supported by a positive correlation between Cu ($r = 0.52$; $P < 0.01$) and Se ($r = 0.79$; $P < 0.01$) intake and calf mineral status. A similar response was reported in a similar pre-weaning mineral supplementation study (Moriel and Arthington, 2013). In that study, calves assigned to a mineral-fortified supplement, using mostly sulfate sources, had negligible supplement consumption during the pre-weaning phase. The authors attributed this response to reduced palatability of the mineral-fortified supplement, which was perhaps due to a similar "metallic taste" proposed in the current study. Additionally, the lack of supplement consumption resulted in no differences in calf

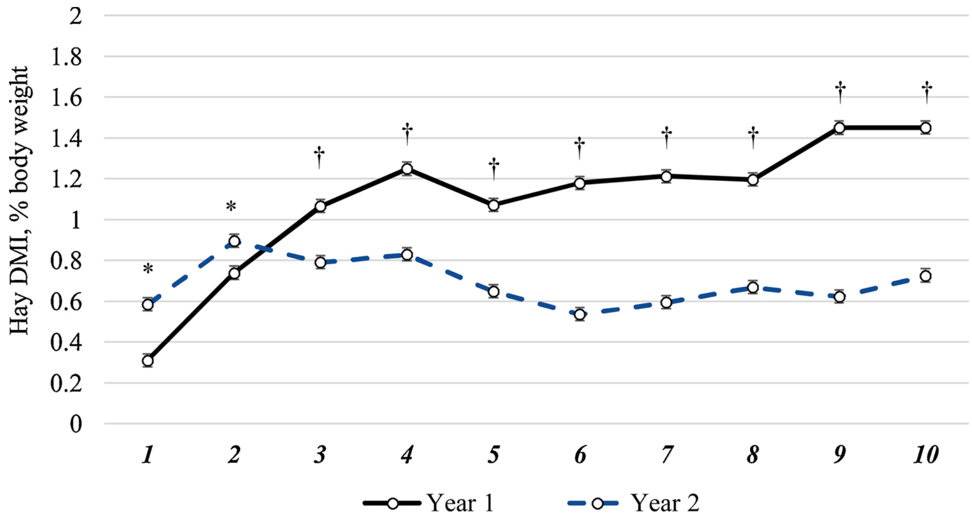

Fig. 4. Hay dry matter intake (**DMI**) as percentage of body weight (**BW**) during the post-weaning phase in Exp. 2. Data is presented as the daily average intake for each interval which consisted of 3 consecutive days. There was a treatment effect ($P < 0.01$) and an interval x year effect ($P < 0.01$). *Calves in year 2 of the study had greater hay DMI as a percentage of BW than calves in year 1 ($P < 0.01$). † Calves in year 1 of the study had greater hay DMI as a percentage of BW than calves in year 2 ($P < 0.01$).

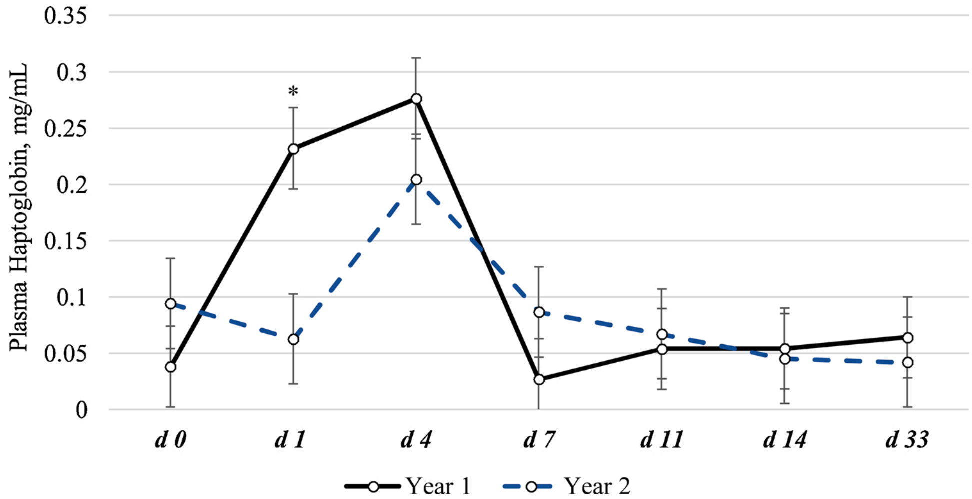

Fig. 5. Plasma haptoglobin (mg/mL) concentrations of calves post weaning in Exp. 2. There was no treatment or treatment x day for plasma haptoglobin concentrations; however, there was a year x day ($P = 0.01$). * Calves in year 1 tended ($P = 0.07$) to have greater plasma haptoglobin concentration than calves in year 2.

mineral status at weaning between mineral-fortified and non-fortified treatments, as observed in year 2 of the current study.

According to NASEM (2016), forage DMI of calves from birth to weaning is approximately 12.5 g/kg of BW. If calculated for the calves in the current study, DMI would be approximately 2.3 kg/d. Therefore, based on NASEM requirements, Cu and Se intake should be 23 and 0.23 mg/d, respectively. In the current study, calves in year 2 were consuming an average of 99 and 4.2 mg of Cu and Se daily but were considered Cu and Se deficient at weaning. Although NASEM recommendations are not specific for young calves, these data indicate that calves from birth to weaning may have a greater requirement for Cu and Se than the suggested requirements for mature beef cattle. Antagonists may also be contributing to findings of the current study. Sulfur is a known antagonist of both Cu and Se (Spears, 2003). Considering a forage DMI of 12.5 g/kg of BW and 0.16 kg/d of mineral-fortified supplement intake, calves would have consumed a diet containing 2.4 g of S/kg (dry matter basis). This amount of S is 60 % greater than the S requirement but it is still less than the maximum tolerable concentration for mature cows (3.0–5.0 g/kg; NASEM, 2016). Although S intake of the calves in the current study is below the maximum tolerable level suggested by NASEM (2016), it is possible that this rate of S consumption for a prolonged period of time is sufficient to disturb Cu and Se metabolism in pre-weaned calves, as it has been demonstrated to negatively impact performance. Zinn et al. (1997) reported that heifers consuming a diet containing 2.5 g of S/kg (dry matter basis) had reduced ADG and decreased longissimus muscle area. However, in year 1, calves had adequate Cu and Se status at weaning despite

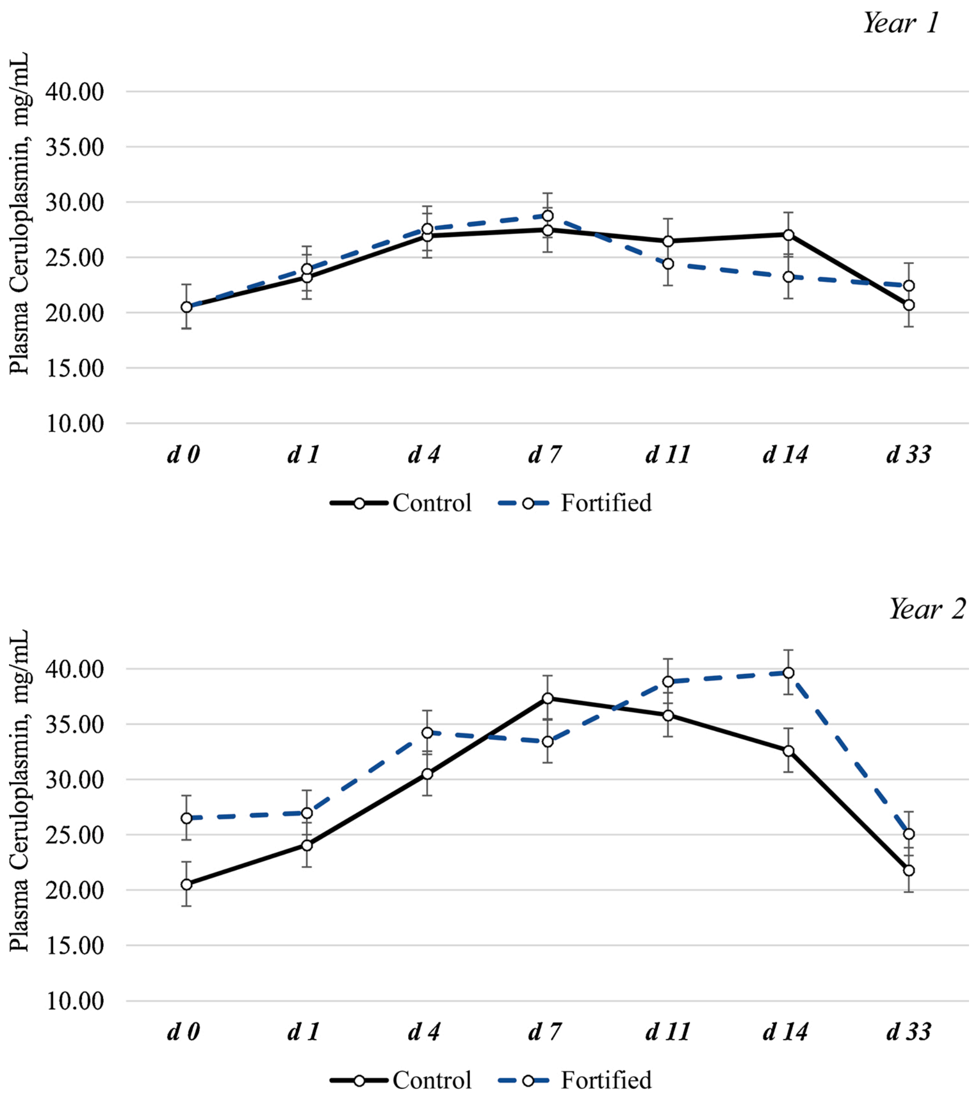

Fig. 6. Plasma ceruloplasmin (mg/dL) concentrations of calves post weaning in Exp. 2 (treatment x day x year; P = 0.01). There was also a day × year (P < 0.01) effect, where plasma ceruloplasmin concentrations were greater on d 7 and 11 in year 2 than year 1.

consumption of diets containing a similar amount of S. These differences in calf mineral status are explained by the large reduction in mineral intake in year 1 vs. 2 (31 and 39 % less Cu and Se intake, respectively). These results illustrate the importance of ensuring adequate supplemental mineral intake of pre-weaned calves when consuming forages and or supplements with high S concentrations.

Calves assigned to the Fortified treatment had greater overall DMI in the final intervals of the 30-d post-weaning evaluation. These results differ from those of Caramalac et al. (2017) where calves provided mineral-fortified supplements pre-weaning had less total DMI than calves provided supplements without mineral fortification. However, their study only measured DMI during the first 15 d after weaning compared to 30 d in the current study. According to Galyean et al. (1995), stressed calves have an altered eating pattern and when given a choice tend to prefer high-concentrate vs. high-roughage diets, which is the opposite response observed in the current study. Calves assigned to the Fortified treatment had greater cortisol concentrations and hay DMI at weaning when compared to calves assigned to the Control treatment. Despite elevated cortisol, calves assigned to the Fortified treatment were not experiencing sufficient stress to elucidate the feeding pattern suggested by Galyean et al. (1995). Cortisol is responsive to an array of insults and simply regrouping of acclimated feedlot cattle, for example, can increase plasma cortisol concentration (Gupta et al., 2005). It is difficult, therefore, to explain the treatment differences observed in the current study, and indeed, previous research have reported variable results. In our previous study investigating pre-weaning supplementation of trace mineral fortified supplements (Caramalac et al., 2017), calves consuming a mineral-fortified supplement tended to have less plasma cortisol concentrations on d 0 (weaning) and 2 after weaning, than calves consuming non-fortified supplements pre-weaning. Marques et al. (2016) evaluated the effects of mineral supplementation during gestation on post-weaning cortisol concentrations in beef calves. In their study, calf cortisol concentrations did not differ at weaning, but was greater on d 3 after weaning in calves born to mineral supplemented vs. unsupplemented cows. The

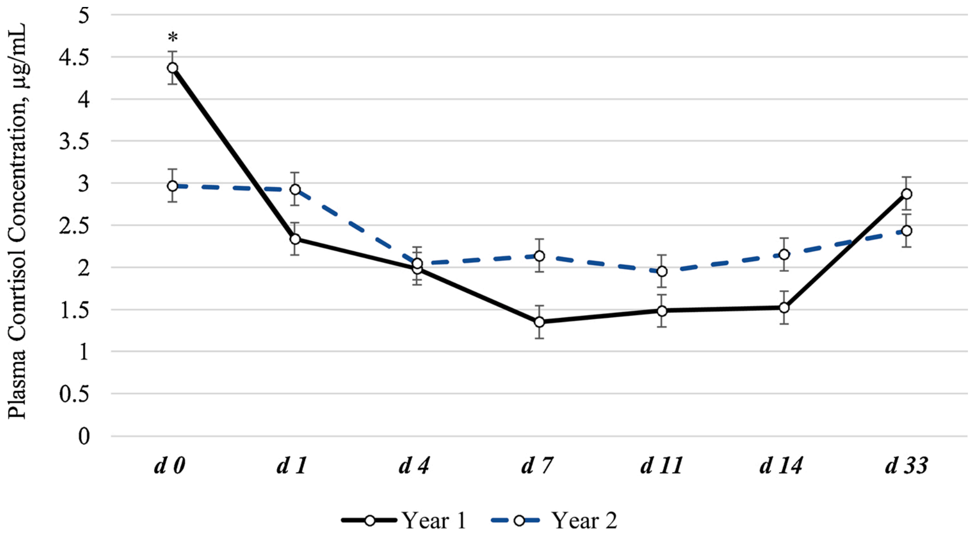

Fig. 7. Plasma cortisol concentrations (µg/mL) of calves post weaning in Exp. 2. There was a treatment effect (P = 0.05) for plasma cortisol concentrations following weaning and a day x year (P < 0.01). * Calves in year 1 had greater plasma cortisol concentration than calves in year 2 (P < 0.01).

authors of that study suggested that trace mineral supplementation may influence steroidogenesis, but this topic deserves further investigation. According to Lofgreen et al. (1975) newly received calves that received alfalfa hay during the first 4 weeks in the feedlot gained less weight and were less efficient during the next 28 days than those that did not receive hay during the first 4 weeks in the feedlot. Although the timing suggested by Lofgreen et al. (1975) is not exactly the same as the current study, the same rationale could be applied, whereas the greater hay consumption observed for calves assigned to the Fortified treatment likely explains the lack of treatment differences for final BW on the current study.

Haptoglobin and ceruloplasmin are both important proteins of the acute phase response. Haptoglobin functions as scavenger for free hemoglobin (Sadrzadeh and Bozorgmehr, 2004), while ceruloplasmin is a Cu-containing protein which carries the majority of Cu in plasma and serves as a ferroxidase (Cousins, 1985). As an acute phase protein, haptoglobin and ceruloplasmin behave similarly in cattle (Cooke et al., 2009) and are responsive to a number of inflammatory conditions as those observed during viral and bacterial challenge (Stabel et al., 1986), handling during weaning (Arthington et al., 2005), transport (Cooke et al., 2013) and vaccination (Arthington et al., 2013). In the current study, a normal acute phase reaction was observed following weaning, with increasing plasma concentrations of ceruloplasmin and haptoglobin; however, mineral fortification of the pre-weaning supplements had no impact on this response. Similarly, Moriel and Arthington (2013) reported that mineral fortification of pre-weaning supplementation did not affect plasma haptoglobin and ceruloplasmin concentrations of calves post weaning. In contrast, Caramalac et al. (2017) reported that calves provided mineral-fortified vs. unfortified supplements pre-weaning had greater peak in concentrations of haptoglobin and ceruloplasmin following weaning. Additionally, Arthington et al. (2014) reported greater plasma haptoglobin and ceruloplasmin concentrations during the 14-d post transport evaluation period for calves receiving trace mineral injections vs. saline, suggesting that the acute phase response may be influenced by trace mineral status of the calves. In the current study, plasma haptoglobin concentrations were greater in year 1 vs 2, which coincides with the greater mineral intake and status observed in the study. However, the same rationale cannot be applied to plasma ceruloplasmin concentrations as greater plasma ceruloplasmin concentrations were observed in year 2 of the study and therefore associated with lower Cu intake. One alternative explanation for greater ceruloplasmin concentration in year 2 would be associated with greater inflammation, which however was not evaluated in this study.

5. Conclusions

In summary, MB supplements, fortified with hydroxychloride sources of Cu, Zn, and Mn, are preferred by calves when compared to MB fortified with sulfate or organic sources of these minerals. Further, the use of mineral-fortified MB supplements is a convenient strategy for delivering minerals to pre-weaned calves, as labor associated with hand-feeding decreases considerably. In the current study, the supplementation of pre-weaned calves with mineral-fortified MB had a positive impact on the mineral status of calves at weaning; however, this response is dependent on adequate supplement intake. These data further imply that the Cu and Se requirements of young calves (birth to weaning) when consuming diets containing S above the requirement, is greater than estimated for other classes of beef cattle. More research in this topic is warranted to unveil mineral requirements of pre-weaned beef calves.

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CRediT authorship contribution statement

J. Ranches: Writing - original draft, Writing - review & editing, Investigation, Resources, Formal analysis, Data curation, Visualization. **R.A. De Oliveira:** Investigation, Writing - review & editing. **M. Vedovatto:** Investigation, Writing - review & editing. **E.A. Palmer:** Investigation, Writing - review & editing. **P. Moriel:** Visualization, Writing - review & editing. **L.D. Silva:** Resources. **G. Zylberlicht:** Resources. **J.S. Drouillard:** Resources, Writing - review & editing. **J.D. Arthington:** Conceptualization, Methodology, Supervision, Project administration.

Declaration of Competing Interest

There are no potential conflicts by the authors.

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